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EVALUATION OF THE ANTIBIOGRAM OF AQUEOUS EXTRACT OF *JATROPHA TANJORENSIS* AND CONVENTIONAL ANTIBIOTICS ON BACTERIAL ISOLATES FROM DIFFERENT BRANDS OF TOMATO PASTE

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ABSTRACT
Tomato (*Lycopersicon esculentum)* is a perishable vegetable widely cultivated and consumed worldwide. The moisture content of tomatoes and mode of packaging has made it prone as source of disease permission vehicles and a major cause of food borne related diseases. The aim of this study was to evaluate the antimicrobial activities of aqueous extract of *Jatropha tanjorensis* and conventional antibiotics on bacterial isolates from different brands of tomato paste. A total of 50 tomato paste samples (10 different brands) were collected from the vendors. The bacterial colonies forming unit were determined using bacterial colony counting chamber. The presence of bacteria contaminants was examined using standard microbiological techniques. The inhibitory capacity of aqueous extracts of *Jatropha tanjorensis* was determined by dispensing the different concentrations of the extracts into the agar wells. Antibiotics susceptibility testing was done using Kirby-Bauer disk diffusion method and multiple antibiotic resistance index (MARI) were determined. The bacterial colony forming unit from the tomato paste samples were revealed to range from 1.0 x 10^4 to 9.2 x 10^4 and 39 samples had visible growth. The samples showed the presence of four different isolates, which include; *Staphylococcus auerus, Escherichia coli, Campylobacter* and *Shigella* species. The most frequently isolated bacteria were *Staphylococcus aureus* at the value of 16(32.0%), followed by *Escherichia coli* 10(20.0%) whereas the least was *Campylobacter* species with 5(10.0%), followed by *Shigella* species 7(14.0%). The inhibition zone diameter produced by the aqueous extracts of *Jatropha tanjorensis* on the bacteria isolates ranged from 38mm at 100mg/ml to 10mm at 25mg/ml. The bacteria isolate showed 31.0% to 100.0% resistance to erythromycin, imipenem and 85.0% to 100.0% susceptible to ciprofloxacin, amikacin, gentamycin, vancomycin. The multidrug resistance index was reported to range from 0.3 to 0.4. This study has reported presence of multidrug resistant bacteria in tomato paste and the promising potential of aqueous extracts of *Jatropha tanjorensis* in inhibiting the growth of these bacteria. Therefore, there is need for proper handling of food items to avoid unnecessary food related disease outbreak among the consumers.

KEYWORD: The samples showed the presence of four different isolates, which include; *Staphylococcus auerus, Escherichia coli, Campylobacter* and *Shigella* species.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the study

Tomato (*Lycopersicon esculentum)* is a perishable vegetable widely cultivated and consumed worldwide (Anie, 2022; Adekalu *et al*., 2016). It is rich in nutrients, vitamins, dietary fibres (Adekalu *et al* 2014; Adekalu *et al*., 2018). It belongs to family Solanaceae, order Solanales and Genus Solanum (Anie, 2019). Due to its nutritive value, taste, affordability, and accessibility, there has been an increase in demand and consumption of both fresh tomatoes and tomato paste by consumers (Anie, 2022). Even though it is a popularly grown vegetable, it suffers huge post-harvest challenges because of its climacteric nature as well as high moisture content, making it susceptible to microbial deterioration and reducing its shelf-life (Mertz *et al*., 2011). However, tomato fruits can be processed into more convenient and shelf stable food products such as tomato powder, tomato juice, tomato concentrates, tomato paste and tomato

purée as a means of preservation and ensuring availability (Fieldhouse, 2013; Gupta *et al.*,2011). In most developing countries, microbial infestation of tomatoes can occur during the harvesting period, post harvesting, handling, storage, transportation, and processing by customers (Bello *et al*., 2016). Centers for Disease Control and Prevention have also reported that another means of bacterial contamination of tomatoes is by exposing them on benches and baskets in the open markets for customers (Adekalu *et al.*, 2016).

For several decades, a significant number of compounds have been identified from tomato fruit when subjected to metabolite evaluation; these compounds represent diverse biosynthetic pathways and covering array of traits (from volatile to highly polar to nonpolar). Some of these biochemical identified comprises of sugars, amino acids, organic acids, fatty acids, hormones, phenolics, alkaloids, and terpenes including carotenoids and various volatile compounds (Kim *et al*., 2019). Previous research has indicated that non-edible parts of the tomato contain a higher content of antimicrobial compounds than edible parts. For instance, tomato leaves include a higher content of antimicrobial metabolites such as chlorogenic acid, caffeic acid, vanillic acid, β-phellandrene, sabinene, α-terpinene, dehydro-tomatine, and α-tomatine than tomato fruits (Kim *et al*., 2014). Tomatoes possess several biochemical components, such as Steroidal alkaloid: Green and unripe parts contain steroid glycosides, in form of glycoalkaloids. Total contents of steroid alkaloids differ from 0.1 up to 0.5 %. It is found in highest amount in fruits and seeds. In the genus Solanum they are important, both ecologically and commercially. The main steroid alkaloids are Solanin and Solasodine. Both consist of an aglycone and are connected mostly with 3 sugar parts like a chain. So, they are called solatrioses (Kobayashi *et al*., 2012).

Some studies have been carried out on bacteria associated with tomatoes and tomato products in some countries. A study carried out on tomato paste has revealed that *Clostridium* specie, *Staphylococcus* specie and *Bacillus* specie were predominant bacteria isolated from both canned and raw tomatoes (Eleyowo and Amusa, 2021). Another study carried out on tomato puree revealed the presence of *Klebsiella* specie, *Proteus mirabilis, Vibrio* species, *and Pseudomonas* specie (Azeez, 2016). In Nigeria, Wogu and Ofuase, (2014) isolated *Bacillus subtils*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Proteus mirabilis,* and *Staphylococcus aureus* from spoilt tomato paste in Benin City. A similar study also revealed high levels of *Staphylococcus* specie, *Bacillus* specie, and *Escherichia coli* in Lagos State, Nigeria.

Antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. For decades, they have fought many infectious diseases and saved millions of lives (Prabakaran and Sujatha 1999). However, the failure of antibiotics due to the rapid spread of new infections and the dramatic emergence of multidrug resistant pathogens has prompted health organizations and pharmaceutical industries around the world to begin screening plants with antimicrobial potential and less toxic than conventional antibiotics for the development of new pharmaceuticals drugs to address both old and new health problems (Sofowora, 2008). Medicinal plants, according to the World Health Organization (WHO), are the best source of a wide range of medications. Many plants have been employed for their antibacterial properties, which are related to phytochemicals produced in the plant's secondary metabolism. Secondary metabolites present in plants include tannins, alkaloids, phenolic chemicals, and flavonoids, which have been shown to have antibacterial activities in vitro. The family Euphorbiceae, is a large family of flowering plants with 300 genera and around 175 species. Most are herbs, but some, especially those distributed in the tropics, are shrubs or trees. They are used by man as food or for ornamental purposes. *Jatropha tanjorensis* belongs to the family 'Euphorbiaceae. Its leaf is commonly consumed as vegetable in many parts of southern Nigeria. It is commonly called 'hospital too far', lapalapa (Iwalewa *et al*., 2005). In southwestern Nigeria, it is called 'Catholic vegetable or Reverend father's vegetable' (Omoregie and Osagie, 2007) possibly because it is grown in the premises of the catholic churches as ornamentals. *J. tanjorensis* has received a lot of attention due to its potential health benefit, availability and affordability (Omobuwajo *et al*., 2011). Its primary use is for fencing, and as medicine for the treatment of diabetics, ulcer, sickle cell anemia, wound infections and for blood buster (Oboh and Masodje, 2009). Phytochemical screening of *Jatropha tanjorensis* leaf revealed that it contains bioactive principles such as alkaloids, flavonoids, tanins, cardiac glycoside, anthraquinones and saponions (Omoregie and Osagie 2007). The pharmacological studies revealed that the plant showed some wide range of biological activities, which include antihypertensive, antioxidant, antimicrobial, antimalarial, hypoglycaemic, hypolipidemic and hematological activities.

MATERIALS AND METHODS

Materials

The equipment/instrument that was used includes: Bunsen burner, autoclave, oven, incubator, weighing balance.

Chemicals and Reagents

The reagents and chemicals that were used in this study includes: sterile water, Dimethyl Sulphur Oxide, normal saline.

Media

The media that were used for this research work includes; Plate Count Agar, Nutrient agar, Mueller Hinton agar, Mannitol Salt agar, Salmonella Shigella agar, MacConkey agar.

Antibiotics
The following antibiotics were used; amikacin (30µg), erythromycin (15µg), gentamycin (30µg), ciprofloxacin (5µg), vancomycin(30µg), imipenem (10µg) and oxacillin (5µg).

Study Area
The study area was in Abakaliki town in Ebonyi State located in south eastern part of Nigeria. It is located 64 kilometers southeast of Enugu. Abakaliki is situated on latitude $6^0$20'N and longitude $8^0$06E.

Sample Collection
Tomato pastes samples of different brands (10 different brands) sold and used in Abakaliki were purchased as samples. Five samples each of the tomato paste were collected from the local markets. The samples were transported to the Microbiology Laboratory of Ebonyi State University, Abakaliki where they were analyzed following standard microbiological techniques (Anie *et al*., 2017).

Analysis of Samples
Ten folds serial dilutions were carried out following standard microbiological procedures, by dissolving One gram each of the tomato paste samples in 9ml of distilled water contained in test tubes labeled according to the brands name. After the dilution, 0.5ml from the dilution factor five (10^5) was transferred to each sterile Petri dish before pouring a sterilized plate count agar. They were all incubated 24 hours at 37^{o}C (AOAC, 2018).

Determination of Aerobic Plate Count
Standard plate count method proposed by AOAC, (2020) was used to determine the total colony count of the samples. Only plates with moderate growth were counted. The average microbial loads of the samples obtained from the different location were expressed as colony forming units per milliliter (Cfu/ml).

Isolation and Identification of Bacteria Isolated from tomato paste Samples.
MacConkey agar, Mannitol Salt Agar and Salmonella – Shigella agar were used for the isolation of bacteria for the purpose of identification. MacConkey agar were used to isolate lactose fermenting Gram negative bacteria, Mannitol Salt agar were used for the selective isolation of salt-tolerant bacteria and Salmonella – Shigella agar were used for the isolation of enteric bacilli particularly *Salmonella* and *Shigella* species. All plates were prepared according to the manufacturers' instruction, inoculated based on microbiological standard method and incubated at 37^0C for 24 hours. Identification of bacteria isolates were based on the standard culture, morphological and biochemical methods (CLSI, 2020).

Gram Staining
A dry smear was made on a slide and fixed by pouring it over flame. The flame smear was covered with crystal violet stains for 30 to 60 seconds, the stain was rapidly washed off with clean water on the slide was tipped off. The smear was covered with lugol's iodine for 30 to 50 seconds, the iodine was washed off with clean water and was decolorized rapidly (few seconds) with acetone-alcohol, it was immediately washed off with clean water, the smear was covered with neutral red for 30 – 60 seconds, the stain was washed off with clean water, the back of the slide was wiped and placed on a draining rack for the smear to air-dry, then the smear was viewed microscopically, with the x100 objective lens and with the immersion oil (Cheesbrough, 2010).

Biochemical Test
Catalase Test
A total of 2 to 3 ml of the hydrogen peroxide solution was poured into test tubes, using a sterile stick or a glass rod (notmchrome wire loop) several colonies of the test organism was removed and immerse in the hydrogen peroxide solution and then immediate bubbling was checked for active bubbling showed positive catalase test, no bubbles showed negative catalase ions (Chessbrough, 2010)

Oxidase test
A filter paper was sealed with the substrate tetramethyl-p-phenylenediamine dihydrichloride with sterile distilled water, and the tested was picked with a wooden or platinum loop and smeared in the filter paper and then observed inoculated area of paper for a colour change to deep blue or purple within 10 – 30 seconds. Oxidase positive organism give blue colour within 5 – 10 seconds and in oxidase negative organism colour does not change (Cheesbrough, 2010).

Indole test
The test organism was inoculated in a bottle containing 3 ml of sterile trypen water, the mixture was inoculated at 35 – 37^{o}C for up to 48 h, indole was tested by adding 0.5 ml of kova's reagent and shaked gently, a red colour was examined in the surface layer within 10 mins red surface layer will indicate positive indole test, no red surface layer indicate negative indole test (Cheesbrough, 2010).

Citrate utilization test (Simmon citrate agar)
Slope of the medium (smmon's citrate agar) was prepared in the bijou bottle as recommended by the manufacturer (stored at 2 – 8^{o}C), the slope was first streaked using a sterile straights wire with a suspension of the test organism and then stab the bun the stabbed slop was inoculated at 35^0C for 48 hours. A bright blue colour was examined in the medium, light blues will show positive citrate test. A change in colour showed negative citrate test of medium (Cheesbrough, 2010).

Methyl Red test
Nutrient broth was prepared and sterilized; the test isolate was inoculated and incubated for 48 hours. 2-3 drops of Methyl Red reagent were added and allowed for 1minutes. The presence of a red colour change showed a positive result**.**

McFarland Turbidity Equivalent Standards
Turbidity standard equivalent of 0.5 McFarland standards were prepared by adding 1.0 ml of concentrated tetraoxosulphate (vi) acid (H_2SO_4) to 99 ml of distilled water. Then 0.5 g of dehydrated barium chloride ($BaCl_2.H_2O$) was dissolved in 50 ml of distilled water in a separate flask. Volumes of 0.6 ml of barium chloride solution were introduced into tetraoxosulphate (vi) acid solution (99.4 ml) in a separate conical flask. It was properly mixed to obtain 0.5 McFarland turbidity equivalent standards. Some portions of the mixed solutions were transferred to test tube and stored at room temperature (28 ^{0}C). This was used to compare with the turbidity of the test organisms prior to antibiotic susceptibility testing (Cheesbrough, 2006).

Standardization of test Bacteria
All the test bacteria were standardized before use by inoculating a loopful of a 24 h culture of the test organism from a nutrient agar slant into test tubes with 5 ml sterile water. Then dilutions using loopful of the test organism and sterile water were carried out in order to obtain microbial population of $1x10^6$ colony forming unit per milliliter (cfu/ml) by comparing it with 0.5 McFarland turbidity standards (CLSI, 2021).

Antimicrobial Susceptibility Testing
The Mueller Hinton agar medium was prepared according to the manufacturer's instructions. An overnight incubated (37^{o}C) pure culture of bacterial isolates in nutrient broth was adjusted to 0.5 McFarland turbidity. The bacteria isolates were inoculated on the plates. Different classes of antibiotics (amikacin, erythromycin, gentamycin, ciprofloxacin, vancomycin, imipenem and oxacillin) were placed on the surface of Mueller-Hinton agar and incubated at (37^{o}C) for 18 h. The inhibition zone diameters (IZD) around each antibiotic disk were measured using a calibrated transparent ruler and recorded in millimeters. A standardized CLSI break point table was used to determine if each bacterium was resistant; intermediate; or susceptible, for analysis, isolates with intermediate or resistant results were merged as resistant (CLSI, 2021).

Extraction of *Jatropha tanjorensis*
The leaves of the *Jatropha tanjorensis* were collected, washed and dried under room temperature. It was then ground into fine powder. A total of 100g were weighed and soaked in 500ml of distilled water for 48 hours. The extracts were obtained by filtering the contents of the flask through a Whatman No.1 filter paper. The liquid extracts were kept in a warm air oven at 50^{o}C for the solvent to evaporate. The dried extract was scraped with a spatula onto a universal bottle for use (Anie *et al*., 2017; Ukpai *et al*., 2016).

Preparation of *Jatropha tanjorensis* Extract
Exactly 7.5g of the dry extracts of *Jatroph tanjorensis* was dissolved in 15mls of DMSO to obtain our stock. Then different concentrations of 100mg/ml, 50mg/ml and 25mg/ml where obtained from the stock by further dilutions. The already prepared Muller-Hinton plates with the isolates were perforated with a cork borer of 6mm deep. The extracts of *Jatropha tanjorensis* of different concentrations were each added on the holes made. The plates were incubated at 37^{o}C for 24 hours and the zones of inhibition observed after 24 hours of incubation. The inhibition zone diameters (IZD) around each well were measured using a calibrated transparent meter ruler and recorded in millimeter.

Determination of Multiple Antibiotic Resistant Index (MARI)
Multiple antibiotic resistance index (MARI) were determined to know the resistant level of the isolates according to the method described by (Fahim *et al*., 2021).

MARI =a/b Where; a=number of antibiotics to which the test isolate showed resistance, b=total number of antibiotics to which the test isolate has been evaluated for sensitivity.

RESULTS
Out of 50 samples of tomato paste tested only 39 had visible growth with colony forming unit ranging from 1.0 x 10^4 to $9.2x10^4$ (table 1). The biochemical and morphological characteristic of the isolates was used to identify the bacteria isolates as Gram positive (*Staphylococcus aureus*) and Gram negative bacteria (*Campylobacter* species, *Escherichia coli* and *Shigella* species) from the tomato paste samples (table 2). The most frequently isolated bacteria from the tomatoes pastes sample was *Staphylococcus aureus* at the value of 16(32.0%), followed by *Escherichia coli* 10(20.0%) whereas the least was *Campylobacter* species with 5(10.0%), followed by *Shigella* species 7(14.0%) (table 3).

The Inhibition zone diameter produced by the aqueous extracts of *Jatropha tanjorensis* on *Staphylococcus aureus* isolated ranged from 34mm at 100mg.ml of the extract to 10mm at 25mg/ml of the same extract (table 4a). The Inhibition zone diameter produced by the aqueous extracts of *Jatropha tanjorensis* on *Escherichia coli* isolated ranged from 10mm at 25mg/ml of the extract concentration to 38mm at 100mg/ml of the same extract (table 4b). The Inhibition zone diameter produced by the aqueous extracts of *Jatropha tanjorensis* on *Campylobacter* species isolated from the tomato paste samples ranged from10mm at 25mg/ml to 30mm at 100mg/ml of the same extract (table 4c). The Inhibition zone diameter produced by the aqueous extracts of *Jatropha tanjorensis* on *Shigella* species isolated ranged from 10mm at 25mg/ml to 32mm at 100mg/ml of the same extract (table 4d).

Antibiotic susceptibility patterns of the bacteria isolated was observed, *S. aureus* and *E. coli* isolates were 100.0% resistance to oxacillin, imipenem and had a susceptibility

range of 80.0% to 100.0% to vancomycin, ciprofloxacin, amikacin, gentamicin. The *Campylobacter* species were 100.0% resistance to oxacillin, 80.0% to imipenem, erythromycin and 100.0% susceptibility to vancomycin, ciprofloxacin, amikacin, gentamicin. *Shigella* species were 100.0% resistance to imipenem and vancomycin (table 5). The result of the multiple antibiotics resistant index observed shows the MAR index to range from 0.3 to 0.4 (table 6).

Table 1: **Microbial Count of the tomato paste samples.**

S/N	Sample code range	Colony count range	CFU/ml range
1	GP1-GP5	7-157	$1.4x10^4$ - $3.14x10^5$
2	ST1-ST5	4-13	$1.2x10^4$ - $8.0x10^4$
3	GO1-GO5	5-110	$1.0x10^4$ - $2.8x10^4$
4	GT1-GT5	3-5	$1.0x10^4$ - $6.0x10^3$
5	FT1-FT5	2-87	$1.2x10^4$ - $8.0x10^4$
6	VT1-VT5	3-46	$1.0x10^4$ - $9.2x10^4$
7	DR1-DR5	3-8	$1.0x10^4$ - $8.0x10^4$
8	T1-T5	4-12	$1.0x10^4$ - $8.0x10^4$
9	TT1-TT5	1-4	$2.0x10^3$ - $8.0x10^4$
10	RT1-RT5	3-9	$1.8x10^4$ - $8.0x10^4$

key: GP = First Brand of Tomato Paste; ST= Second Brand of Tomato Paste; GO =Third Brand of Tomato Paste; GT= Fourth Brand of Tomato Paste; FT= Fifth Brand of Tomato Paste; VT= Sixth Brand of Tomato Paste; DT= Seventh Brand of Tomato Paste; T= Eighth Brand of Tomato Paste; TT= Ninth Brand of Tomato paste; RT=Tenth Brand of Tomato paste; CFU=Colony Forming Unit; NG= No Growth

Table 2: Morphological and Biochemical characteristic of bacterial isolates from tomato paste samples.

S/NO	MORPHOLOGY	CO	IN	MR	OX	CAT	GS	ORGANISM ISOLATED
1	Golden yellow, cocci shape (MSA)	+	-	-	-	+	+	*Staphylococcus aureus*
2	Greyish rod shape (MacConkey)	-	-	-	+	+	-	*Campylobacter* specie
3	Pink, rod shape (SSA)	-	-	+	-	+	-	*Shigella specie*
4	Pink rod shape (MacConkey)	_	+	-	-	+	-	*Escherichia coli*

Key= CO=Coagulase; GS= Gram staining; IN=Indole; MR=Methyl Red Test; OX= Oxidase; CAT=Catalase; MSA=Mannitol Salt Agar; SSA=Salmonella Shigella Agar.

Table 3: Frequency of bacterial isolates from tomato paste samples.

S/No	No. of samples	Isolates identified	Percentage frequency (%)
1	50	*Staphylococcus aureus*	16 (32.0)
2	50	*Escherichia coli*	10 (20.0)
3	50	*Shigella* species	7 (14.0)
4	50	*Campylobacter* species	5 (10.0)

Table 4a: Inhibition zone diameter of *Staphylococcus aureus* isolated from the tomato paste samples against aqueous extracts of *Jatropha tanjorensis.*

Isolates	Inhibition zone diameter and extract concentration (mg/ml)		
	100mg/ml	50mg/ml	25mg/ml
S1	30	22	20
S2	30	22	20
S3	30	27	12
S4	32	25	25
S5	28	30	20
S6	30	Nil	22
S7	30	27	18
S8	24	20	15
S9	26	20	17
S10	34	22	18
S11	28	20	20
S12	30	25	18
S13	25	20	20

S14	28	23	10
S15	30	25	20
S16	28	20	14

Key: S1 to S16 = *Staphylococcus aureus* isolated from tomato paste samples

Table 4b: Inhibition zone diameter of *Escherichia coli* isolated from tomato paste samples against aqueous extracts of *Jatropha tanjorensis.*

Isolates	Inhibition zone diameter and extract concentration (mg/ml)		
	100mg/ml	50mg/ml	25mg/ml
E1	28	28	14
E2	24	28	Nil
E3	25	20	15
E4	28	24	20
E5	29	26	20
E6	30	22	12
E7	28	30	20
E8	25	22	10
E9	30	25	Nil
E10	38	20	15

Key: E1 to E10 = *Escherichia coli* isolated from tomato paste samples

Table 4c: Inhibition zone diameter of *Campylobacter* species isolated from tomato paste samples against aqueous extract of *Jatropha tanjorensis.*

Isolates	Inhibition zone diameter and extract concentration (mg/ml)		
	100mg/ml	50mg/ml	25mg/ml
C1	25	15	10
C2	26	20	15
C3	30	25	20
C4	30	25	15
C5	27	24	20

Key: C1 to C5 = *Campylobacter* species isolated from tomato paste samples

Table 4d: Inhibition zone diameter of *Shigella* species isolated from tomato paste samples against aqueous extract of *Jatropha tanjorensis.*

Isolates	Inhibition zone diameter and extract concentration (mg/ml)		
	100mg/ml	500mg/ml	25mg/ml
Sh1	25	20	10
Sh2	32	28	22
Sh3	25	22	10
Sh4	25	22	18
Sh5	26	22	10
Sh6	26	23	15
Sh7	27	26	20

Key: Sh1 to Sh7 = *Shigella* species isolated from tomato paste samples

Table 5: Antibiotic susceptibility profile of the bacteria isolates.

Antibiotic used	*S. aureus* (n=16)		*E. coli* (n=10)		*Campylobacter* sp (n=5)		*Shigella* sp (n=7)	
	R	S	R	S	R	S	R	S
OX	16(100.0)	0(0.0)	10(100.0)	0(0.0)	5(100.0)	0(0.0)	1(15.0)	6(85.0)
IM	16(100.0)	0(0.0)	10(100.0)	0(0.0)	4(80.0)	1(20.0)	7(100.0)	0(0.0)
VA	3(19.0)	13(81.0)	2(20.0)	8(80.0)	0(0.0)	5(100.0)	7(100.0)	0(0.0)
CIP	1(16.0)	15(94.0)	1(10.0)	9(90.0)	0(0.0)	5(100.0)	1(15.0)	6(85.0)
AK	0(0.0)	16(100.0)	0(0.0)	(100.0)	0(0.0)	5(100.0)	0(0.0)	7(100.0)
CN	0(0.0)	16(100.0)	2(20.0)	8(80.0)	0(0.0)	5(100.0)	1(15.0)	6(85.0)
E	5(31.0)	11(69.0)	7(70.0)	3(30.0)	4(80.0)	1(20.0)	5(72.0)	2(28.0)

Key: n= Number of isolates; R= Resistant; S=Sensitive; OX=Oxacillin; IM=Imipenem; VA=Vancomycin; CIP=Ciprofloxacin; AK=Amikacin CN=Gentamycin E=Erythromycin

Table 6: Multiple antibiotics resistant index.

ISOLATES	MARI
Staphylococcus aureus	0.3
Escherichia coli	0.4
Campylobacter species	0.4
Shigella species	0.4

Key: MARI= Multiple Antibiotics Resistance Index

DISCUSSION

Tomato products are essential source of some nutrients such as vitamin A, vitamin C, fiber, potassium, and very important herbaceous plant. This research evaluated the antimicrobial activities of aqueous extract of *Jatropha tanjorensis* and conventional antibiotics on bacterial isolates from different brands of tomato paste. Out of the 50 sachets of tomato paste tested only 39 had visible growth. The different samples of tomato paste purchased from their vendors showed varying bacterial count. The bacterial count reveals that the instant tomato paste obtained from their vendors had a bacteria colony within the range of $1.0x10^4$ to $9.2x10^4$. This result agrees with Annie, (2022) that carried out similar work on fresh tomatoes and tomato paste in delta state, south western Nigeria, in his work he reported microbial load on fresh tomatoes to be 7.4 $x10^4$ total coliform counts and tomato paste was 3.6 x 10^4 to 5.0 $x10^4$. The total bacterial count of the tomatoes paste sample showed that there were variations at different samples. The microbial load observed in the tomatoes paste samples indicated that the tomatoes pastes were contaminated with microbes of medical importance. The microbial load from the analyzed samples was quite higher in value. The microbial load of GP (First brand of tomato paste) among the analyzed brands of tomatoes paste was higher compared to other tomatoes paste closely followed by GO (Third brand of tomato paste). This could be due to the fact that tomatoes contain nutrient that can support the growth of microorganisms as well as the storage condition. This agrees with the report of Romero *et al*. (2014) that noted that using unhygienic environment and water for processing of food are responsible for food contamination. This finding is also in line with the study conducted by (Chukwu *et al*., 2008) who isolated several bacterial and fungal from tomato fruits. Tomatoes basically contain all the nutrients necessary for microbial growth and metabolism, making it susceptible to microbial contamination. On the other hand, the presence of microbial growth in some tomatoes paste could be attributed to the level of heating during processing of the tomatoes paste.

A total of four different bacteria isolates were identified from the tomato paste analyzed and they include; *Staphylococcus auerus, Escherichia coli, Shigella* and *Campylobacter* species. The most frequently isolated from the sample was *Staphylococcus aureus* at the value of 16(32.0%), followed by *Escherichia coli* 10(20.0%), *Shigella* species 7(14.0%), whereas the least was *Campylobacter* species with 5(10.0%) prevalence. This report aligned with the observation of Anie, (2022) and that of Adekalu *et al.,* (2016) were they reported different types of bacteria which includes; *Citrobacter* species (31.25%), *Streptococcus* species (12.5%) and *Bacillus subtilis* (12.5%) to contaminate tomatoes pastes. In a similar study by Wogu and Ofuase (2014), several bacterial (*Staphylococcus auerus, Escherichia coli, Citrobacter* species, *Streptococcus* species, *Bacillus subtilis, Shigella* and *Campylobacter* species) were identified as being associated with the spoilage of tomato fruits. The result of this present study also agrees with the report of Kwack and Chun (2014) who reported that freshly prepared food and vegetable product could be contaminated with diverse microbes especially when prepared with unsterile water.

This study also suggested that the presence of microbial contaminates in tomato paste could also have been as a result of the exposure of the tomatoes to the atmosphere during the period of preparation from handlers and is in line with the report of previous researcher (Chime *et al*., 2017). Exposure of the tomatoes to unhygienic condition during preparation, lack of good preservation method and standard equipment for processing the product is another notable factor in sample contamination. The presence of these microorganisms may also be attributed to micro flora or due to spoilage microorganisms that gained their entrance into the tomatoes after preparation. Another study stated that *Staphylococcus aureus*, and some enteric bacteria gained access to contaminate tomato paste via the use of contaminated water and poor preservation methods (Chukwura and Majekwu, 2002) and contamination from intestine of slaughtered animals which can possibly come in contact with the raw material used for the preparation of the tomato paste sample (Ghosh, 2009). *Staphylococcus* species was reported to cross contaminate from tomatoes handlers during processing, since it is a normal flora of the skin, raw tomatoes is usually carried on the body by harvesters in Nigeria due to lack of proper harvesting training (Onuorah and Orji, 2015). However, the relatively high moisture content in tomatoes makes it highly susceptible to spoilage by microorganisms, which of course is a public health risk.

Previous study has stated that different brands of tomatoes paste can harbor presence of public health important microorganisms such as *E. coli* and *Shigella* species, which usually can be found in soil, vegetation and surfaces of plants, humans and animals (Field, 2002). The major sources of microbial contamination of tomatoes and tomatoes paste appear to be handling by vendors and the use of contaminated water and equipment. Therefore, control of tomatoes contamination can be achieved if aseptic techniques are employed during preparation and preservation. However, tomatoes need to be pasteurized in a very hot water before use for consumption, as prescribed by health organizations who stated that fresh tomatoes for consumption is not free from microorganisms due to contamination from the environment and processing if not properly pasteurized.

The vendors sell with their bare hands and also simultaneously handle currency as they take money from the buyers, a common practice implicated in introducing pathogens into the food (Kim *et al*., 2019; Collen, 2021). Prevalence rate of bacterial isolates from the samples revealed the possibility of poor method of handling as these organisms are reported to be associated with skin, the environment and water (Martins *et al*., 2009).

The antibiogram of the bacteria isolate showed 31.0% to 100.0% resistance to erythromycin, imipenem and 85.0% to 100.0% susceptible to ciprofloxacin, amikacin, gentamycin, vancomycin. When compared with the aqueous extracts of *Jatropha tanjorensis* the inhibition zone diameter produced by the bacteria isolates ranged from10mm at 25mg/ml to 38mm at 100mg/ml. This suggested that *Jatropha tanjorensis* aqueous extract has a wide range of antimicrobial inhibitory activities against the tested bacteria isolates. This observation is in accordance with report of previous researcher (Ibezim *et al*., 2011). The leaves of *Jatropha tanjorensis* possess antimicrobial properties as observed in the examination carried out by Tarawneh et al. (2010) who reported that the antimicrobial potency of methanol extract of *J. tanjorensis* is attributed to the innate presence of bioactive compounds which includes tannins, flavonoids, alkaloids, anthraquinone, terpenoid, fixed oil, carbohydrate and cardenolide. Other studies has proved that the extracts of *Jatropha tanjorensis* contains Saponins, terpenoid, flavonoids and tannins which are major antimicrobial properties (Chen *et al*., 2013; Dong *et al*., 2017; Vargas *et al*., 2018).

Multidrug resistance bacteria isolates was reported with MAR index ranging from 0.3 to 0.4 and all the isolates were tested against the *J. tanjorensis* extract. The aqueous extract of *J. tanjorensis* showed different levels of potency against all the tested multi drug resistant bacteria isolates. The aqueous extract showed a greater antimicrobial potency against *Escherichia coli* with a zone of inhibition ranging from 38mm at 100mg/ml to 10mm at 25mg/ml; *Shigella* species, 32mm at 100mg/ml to 10mm at 25mg/ml; *Staphylococcus auerus*, *Campylobacter* species, 30mm at 100 mg/ml to 10mm at 25mg/ml. This is similar to the report that stated that *Jatropha tanjorensis* aqueous extract antimicrobial potency against *Enterobacter clocae* with a zone of inhibition of 17mm at a concentration of 100mg/ml, 10mm at 80mg/ml and 8mm at 50 mg/ml and it also had potent inhibitory effect against *Escherichia coli* and *Salmonella typhimerium* (Asuk *et al.,* 2015). Another study has also reported antimicrobial potential of *Jatropha tanjorensis* extract against multidrug resistance bacteria isolates to range from 20.0% to 80.0% depending on the concentration (Khanan *et al*., 2009). The uses of medicinal herbs in disease management have immense benefit because plant medicine is relatively safe, more affordable and sometimes offers better therapeutic value than synthetic drugs (Asuk *et al.,* 2015).

CONCLUSION

This study reported the bacterial colony forming unit from the tomato paste samples to range from 1.0 x 10^4 to 9.2 x 10^4 and 39 out of the 50 samples analyzed had visible growth. The samples showed the presence of four different isolates, which include; *Staphylococcus auerus, Escherichia coli, Campylobacter* and *Shigella* species. The most frequently isolated bacteria was *Staphylococcus aureus* at the value of 16(32.0%), followed by *Escherichia coli* 10(20.0%) whereas the least was *Campylobacter* species with 5(10.0%), followed by *Shigella* species 7(14.0%). The inhibition zone diameter produced by the aqueous extracts of *Jatropha tanjorensis* on the bacteria isolates ranged from 38mm at 100mg/ml to 10mm at 25mg/ml. The bacteria isolate had 31.0% to 100.0% resistance to erythromycin, imipenem and 85.0% to 100.0% susceptible to ciprofloxacin, amikacin, gentamycin, vancomycin. The multidrug resistance index was reported to range from 0.3 to 0.4. This study has reported presence of multidrug resistant bacteria in tomato paste and the promising potential of aqueous extracts of *Jatropha tanjorensis* in inhibiting the growth of these bacteria.

Recommendation

Improvement in the microbial quality of fresh tomatoes and tomatoes paste is very important and adequate steps must be taken to prevent contamination and spoilage by microorganisms. Adequate harvesting, processing, preservation methods is highly recommended to mitigate microbial contamination and spoilage of tomatoes paste. The tomatoes paste can be persevered at low moisture content which will discourage microbial survives proliferation and hence its spoilage and potential to become less health risk to human. The organisms isolated from the tomatoes paste indicate that the standard of preparation and preservation is poor over the years and facilities used for preparation are not sterile. Aseptic techniques should be adequately employed in the food industries so as to reduce microbial load of its products for safe consumption and thus prevent food-borne diseases or infections. Standard personal hygiene should be observed by food handlers and adequate preparation of these said food items.

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